

Sexual Dimorphism, Female Reproductive Characteristics and Egg Incubation in an Oviparous Forest Skink (*Sphenomorphus incognitus*) from South China

Li MA^{1,2}, Jianchi PEI², Cuntong ZHOU^{2,3}, Yu DU^{2,4}, Xiang JI² and Wen SHEN^{1*}

¹ School of Sports and Health, Hangzhou Normal University, Hangzhou 310036, Zhejiang, China

² Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing 210023, Jiangsu, China

³ College of Ecology, Lishui University, Lishui 323000, Zhejiang, China

⁴ Hainan Key Lab for Herpetology, College of Tropical Biology and Agronomy, Hainan Tropical Ocean University, Sanya 572022, China

Abstract We studied sexual dimorphism and female reproduction in an oviparous forest skink (*Sphenomorphus incognitus*) from South China. We incubated eggs under five thermal regimes (22, 25, 28, 25 ± 3 and 27 ± 5 °C) to examine the effects of constant versus fluctuating temperatures on incubation length and hatchling morphology. In our sample the largest male and female were 110 mm and 108 mm snout-vent length (SVL), respectively. Adult males and females did not differ in mean SVL; adult males were larger in head size (both length and width), longer in fore- and hind-limb lengths and shorter in abdomen length than females of the same SVL. Accordingly, we conclude that *S. incognitus* is a sexually monomorphic species in terms of SVL but shows sexual dimorphism in head size, abdomen length and appendage length. Females laid a single clutch of 3–10 eggs per breeding season from early May to mid-August, with larger females generally laying more (but not always larger) eggs per clutch than did smaller ones. Embryonic stages at laying ranged from Dufaure and Hubert's (1961) stage 31 to 32, with a mean stage of 31.3. The positive relationship between clutch mass and female SVL was not significant. The offspring size-number trade-off does not exist in *S. incognitus*, as revealed by the fact that egg mass was independent of relative fecundity. Incubation length decreased as temperature increased, and stable temperatures resulted in delayed hatching. Hatchlings incubated under the five thermal regimes did not differ from each other in any examined trait, suggesting that *S. incognitus* is among oviparous reptilian species where incubation temperature has no role in modifying hatchling morphology as long as eggs are not exposed to extreme temperatures for prolonged periods of time.

Keywords egg, hatchling morphology, incubation length, reproduction, Scincidae, sexual dimorphism

1. Introduction

Forest skinks of the reproductively bimodal genus *Sphenomorphus* Fitzinger, 1843 occur in South-East Asia, Asia, Indochina and Central America (Linkem *et al.*, 2011). Of some 145 currently recognized *Sphenomorphus* species (Linkem *et al.*, 2011), six (*S. courcyanus*, *S.*

incognitus, *S. indicus*, *S. maculatus*, *S. taiwanensis* and *S. tonkinensis*) can be found in China, with *S. taiwanensis* endemic to Taiwan Province of the country (Huang, 1999; Nguyen *et al.*, 2011, 2012). Despite its wide geographic distribution, high species diversity and the fact that it is morphologically, zoogeographically and taxonomically well known, the ecology and biology of the genus *Sphenomorphus* remain poorly studied. Several investigators have studied sexual dimorphism and female reproduction but, to the best of our knowledge, they only reported descriptive data for five species (*S. incognitus*:

* Corresponding author: Ms. Wen SHEN, a technician from School of Sports and Health, Hangzhou Normal University, Hangzhou, China, with her research focusing on physiological ecology of animals.

E-mail: 2542811545@qq.com

Received: 7 February 2018 Accepted: 1 June 2018

Huang, 2010; *S. indicus*: Huang 1996; Ji and Du, 2000; Ji *et al.*, 2006; *S. jagori*: Auffenberg and Auffenberg, 1989; *S. maculatus*: Huang, 1999; *S. taiwanensis*: Huang, 1997, 1998). Detailed data on female reproductive traits do not exist for all these species except for *S. indicus* (Ji and Du, 2000; Ji *et al.*, 2006). For example, ten female *S. incognitus* (Huang, 2010), nine female *S. taiwanensis* (Huang, 1997) and a unknown number of female *S. jagori* (Auffenberg and Auffenberg, 1989) were measured for fecundity (clutch size), but in none of these species were egg mass and reproductive output (clutch mass) documented.

Sphenomorphus incognitus studied here ranges from Southern-Central China (Anhui, Fujian, Guangdong, Guangxi, Hainan, Hubei, Taiwan, Yunnan and Zhejiang) to North Vietnam (Huang, 1999; Lau, 2005; Nguyen *et al.*, 2012; Tang and Huang, 2014; Chen *et al.*, 2017). This medium sized (up to 107 mm snout-vent length, SVL), oviparous terrestrial skink shows a preference for stream habitats, forest edges and riverbeds (Huang, 1999; Nguyen *et al.*, 2012). The skink is morphologically similar to *S. indicus*, its viviparous congener, and this similarity contributes to the confusion about taxonomic identity, habitat use and geographic distribution of these two species (Chen *et al.*, 2017). Previous studies presented very limited descriptive data for *S. incognitus* from mainland China (Huang, 1999), and a bit more detailed data for a population on Lanyu Island, Taiwan, China (Huang, 2010). From Huang's (2010) study on *S. incognitus* from Lanyu Island we know the following. First, males are larger in terms of linear body size (SVL) and thus *S. incognitus* is among species that show male-biased sexual size dimorphism (SSD). Second, females exhibit spring and summer vitellogenesis and lay eggs from March to July. Third, females lay 3–6 eggs per clutch, with clutch size being independent of female SVL. Here, we presented data for *S. incognitus* from South China. Based on morphological measurements taken for adults in the field and clutches laid in the laboratory, we studied sexual dimorphism in body size and shape, female reproduction and egg incubation. Our aims were: (1) to show sexual dimorphism in several morphological characters (body size, head size, head width, abdomen length, and fore- and hind-limb lengths) likely to be associated with reproductive success and performance; (2) to investigate the relationships among egg size (and thus hatchling size), clutch size and female size; and (3) to examine the effects of constant versus fluctuating temperatures on incubation length and hatchling morphology.

2. Materials and Methods

We collected 263 adult skinks (92 females and 171 males) larger than 80 mm SVL in three consecutive years between 2013 and 2015 from Guangzhou, Wuzhishan and Zhaoqing in South China. Most of these skinks (65 females and all males) were released at their point of capture following the collection of morphological data. Measurements taken for each skink with Mitutoyo digital calipers included SVL, abdomen length (AL, between the insertion points of the fore- and hind-limbs), head length (HL, from the snout to the anterior edge of tympanum) and head width (HW, the posterior end of the mandible) (Sun *et al.*, 2012). Of the 263 adults, 123 (42 females and 81 males) were also measured for fore-limb length (FLL, humerus plus ulna) and hind-limb length (HLL, femur plus tibia) (Ji *et al.*, 2007).

We palpated all adult females in the field and transported 27 females with enlarged follicles to our laboratory in Nanjing, where they were individually housed in $540 \times 400 \times 320 \text{ mm}^3$ plastic cages placed in a room inside which temperatures varied from 20 °C to 28 °C. All cages had a substrate consisting of moist soil (~150 mm depth) covered with cobblestones, grass and fallen leaves, and females were able to regulate body temperature using natural sunlight. Mealworms (*Tenebrio molitor*), house crickets (*Achetus domesticus*), cockroaches (*Blaptica dubia*) and water enriched with vitamin and minerals were provided or refreshed daily.

Females laid a single clutch of eggs between early May and mid-August. We checked the cages at least thrice daily for freshly laid eggs after the first female laid eggs, thereby collecting, weighing and measuring (for length and width) eggs always less than 6 h post-laying. Post-oviposition females were weighed and measured for SVL. Of the 27 females, two were excluded from analyses because they laid unfertilized eggs or abnormal eggs with condensed yolk. We calculated relative clutch mass (RCM) by dividing clutch mass by the post-oviposition female mass (Shine, 1992). To account for the influence of variation in female size on fecundity, we calculated relative fecundity by using the residuals derived from the regression of clutch size on female SVL (Olsson and Shine, 1997).

We collected 142 fertilized egg, of which eight, each from one of eight clutches, were used to identify the Dufaure and Hubert's (1961) stage of embryonic development at laying. The remaining eggs were individually placed into covered plastic jars (50 ml) with moist vermiculite at -12 kPa (Ji and Braña, 1999). All

incubating egg were 2/3 buried in the substrate, with the surface near the embryo exposed to air inside the jar. Eggs from the same clutch were assigned as equally as possible among five incubators (Binder, Germany): three set at 22, 25 and 28 °C, respectively; the other two set at 25 ± 3 °C and 25 ± 5 °C, respectively. Thermal fluctuations were maintained at 12 h (+) and 12 h (−) and were confirmed with Tinytalk temperature loggers (Gemini Pty, Australia) placed inside jars. We rotated jars at 4-d intervals to minimize the influence of thermal gradients. Substrate water potential was adjusted at 4-d intervals by weighing jars. Incubation length was defined as the time between laying and pipping. Upon emergence, hatchlings were collected, weighed and measured for SVL, AL, HL and HW.

We used linear regression analysis to examine if the relationship between a selected pair of dependent and independent variables was significant. We calculated regression residuals of an examined morphological variable (AL, HL, HW, FLL, or HLL) against SVL, and then used one-way ANOVA to see if the variable differed between male and female adults. Data on egg size, incubation length and hatchling morphology from the same clutch were pooled to avoid pseudo-replication. We used G-test and one-way ANOVA to see if eggs incubated under different thermal regimes differed in hatching success, mean mass at laying and mean incubation length. We used one-way ANCOVA to test for slope homogeneity of regressions lines and to see if hatchlings from eggs assigned to different treatments differed morphologically after accounting for egg mass at laying. Prior to parametric analyses, all data were tested for normality using the Kolmogorov-Smirnov test, and for homogeneity of variances using Bartlett's test. All statistical procedures were performed in Statistica 8.0 (StatSoft; Tulsa, OK, USA), and statistical significance was assumed at $P < 0.05$. Values are presented as mean \pm standard error (SE) and range.

3. Results and Discussion

3.1. Sexual dimorphism The largest male and female were 110 mm and 108 mm SVL, respectively. Both values are greater than the maximal sizes ever reported for *S. incognitus* from mainland China (107 mm SVL; Huang, 1999) and Taiwan, China (94 mm SVL; Huang, 2010). The mean SVL did not differ between male (97 ± 0.5 mm) and female (96 ± 0.7 mm) adults (ANOVA; $F_{1, 261} = 0.45$, $P = 0.50$; Figure 1), suggesting that *S. incognitus* from South China is sexually monomorphic in terms of adult

body size (SVL). This pattern of SSD differs from male-biased SSD reported for *S. incognitus* from Taiwan, China (Huang, 2010), and it also does not support the hypothesis that lizards on islands are more likely to exhibit male-biased SSD (Hernández-Salinas *et al.*, 2014). Much more adults were measured in this study (92 females and 171 males) than in the earlier one (43 females and 45 males; Huang, 2010), thus allowing more accurate determination of SSD.

The evolution and maintenance of a given pattern of SSD often result from sexual differences in reproductive success relating to adult body size (Cooper and Vitt, 1989; Hews, 1990; Mouton and Van Wyk, 1993; Reeve and Fairbairn, 2001; Cox *et al.*, 2003). Within scincid lizards, selection through male contest competition is the key factor for male-biased SSD in *Plestiodon chinensis* (Lin and Ji, 2000), *Plestiodon elegans* (Du and Ji, 2001; Zhang and Ji, 2004) and *Eutropis multifasciata* (Ji *et al.*, 2006), whereas selection on fecundity or reproductive output is the main cause for increased female size in *S. indicus* (Ji and Du, 2000), *Scincella modesta* and *Scincella reevesii* (Yang *et al.*, 2012). Sexual size monomorphism (SSM) often occurs in species where these two selective forces cancel each other out and has been documented in a wide range of lizard taxa. In lizard species so far studied in China, SSM has been documented in *Calotes versicolor* (Ji *et al.*, 2002), *Eremias argus* (Chen *et al.*, 2015), *Eremias brenchleyi* (Xu and Ji, 2003), *Eremias multiocellata* (Li *et al.*, 2006), *Japalura splendida* (Lin, 2004), *Phrynocephalus frontalis* (Qu *et al.*, 2011), *Phrynocephalus grumgrzimailoi* (Liu and Shi, 2009), *Phrynocephalus guinanensis* (Ji *et al.*, 2009), *Shinisaurus crocodilurus* (He *et al.*, 2011), *Takydromus septentrionalis* (Ji *et al.*, 1998; Zhang and Ji, 2000) and *Takydromus sexlineatus* (Xu *et al.*, 2014).

The rates at which HL (Figure 2a) and HW (Figure 2b) increased with SVL were greater in adult males (ANCOVA for slope homogeneity, both $P < 0.001$), and the rates at which AL (Figure 2c), FLL (Figure 2d) and HLL (Figure 2e) increased with SVL did not differ significantly between the sexes (ANCOVA for slope homogeneity; all $P > 0.09$). The mean values of residuals from the regressions of HL, HW, FLL and HLL on SVL were greater in adult males (ANOVA; all $P < 0.0001$), whereas the mean value of residuals from the regressions of AL (ANOVA; $F_{1, 261} = 64.20$, $P < 0.0001$) on SVL was greater in adult females. The greater relative head size in males and the greater relative abdomen size in females are the rule in nearly all lizard lineages (Olsson *et al.*, 2002; Cox *et al.*, 2003; Kratochvíl *et al.*, 2003;

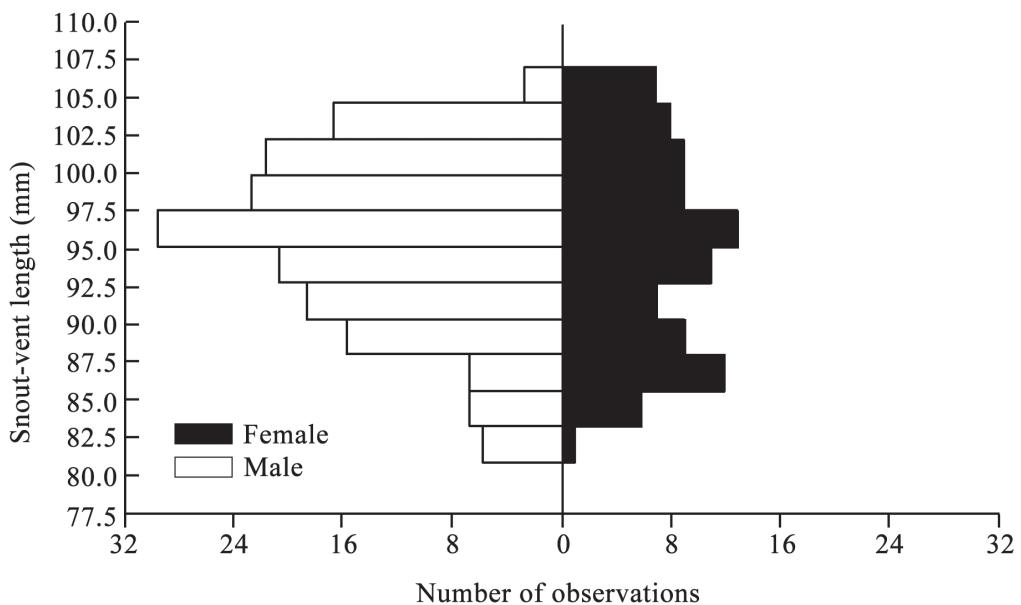


Figure 1 Frequency distributions of SVL of adult *Sphenomorphus incognitus* (92 females and 171 males), showing sexual size monomorphism.

Pincheira-Donoso and Tregenza, 2011; Sun *et al.*, 2012; see also Huang, 1996). It is therefore not surprising that *S. incognitus* shares these features. Head size (both length and width) and abdomen length are sexually dimorphic largely because these traits are directly linked to the reproductive role of each sex (Bulté *et al.*, 2008), although in some species the greater relative head size in males may also have a secondary role in reducing intersexual resource competition by amplifying food niche divergence between the sexes (Braña, 1996; Lin and Ji, 2000; Zhang and Ji, 2000, 2004). Sexual dimorphism in appendage (limb) length has been poorly known. Like *Phrynocephalus przewalskii* (Zhao and Liu, 2014) and *S. incognitus* from Taiwan, China (Huang, 2010), *S. incognitus* from South China shows male-biased sexual dimorphism in appendage length.

3.2. Female reproductive characteristics Table 1 shows female reproductive traits of *S. incognitus* from South China. Females laid a single clutch of 3–10 eggs per breeding season from early May to mid-August, with the egg-laying season being about three months longer than that (from March to July) reported for *S. incognitus* from Taiwan, China (Huang, 2010). Clutch size was positively related to female SVL ($r^2 = 0.18$, $F_{1,23} = 5.09$, $P = 0.034$), suggesting that, as in most other lizard species (Ramírez-Bautista *et al.*, 2017), female size is an important determinant of fecundity in *S. incognitus*. Such a relationship between clutch size and female SVL was nonetheless not statistically significant in *S. incognitus*

from Taiwan, China (Huang, 2010). The mean clutch size was greater in South China (5.2; Table 1) than in Taiwan, China (4.0; Huang, 2010). This difference could be in part due to the fact that females of this study (81–108 mm SVL; Table 1) were larger than those studied in Taiwan, China (73–87 mm SVL; Huang, 2010), as *S. incognitus* is among species where larger females are more fecund than smaller ones. Egg mass and clutch mass had never been examined in *S. incognitus*. In this study, we found that neither clutch mass ($r^2 = 0.12$, $F_{1,23} = 3.23$, $P = 0.085$) nor egg mass ($r^2 = 0.04$, $F_{1,23} = 0.99$, $P = 0.33$) was significantly related to female SVL. These findings suggest that female size is not an important determinant of reproductive output or investment per offspring in *S. incognitus*. Egg mass was independent of relative fecundity ($r^2 = 0.03$, $F_{1,23} = 0.64$, $P = 0.43$), suggesting that, as in *Eutropis longicaudata* (Sun *et al.*, 2012) and *S. modesta* (Yang *et al.*, 2012), the egg size-number trade-off does not exist in *S. incognitus*.

Among oviparous skinks so far studied in mainland China, the mean RCM was smaller in *S. incognitus* (0.25; Table 1) than in *S. modesta* (0.72; Yang *et al.*, 2012), *E. longicaudata* (0.34; Sun *et al.*, 2012), *P. chinensis* (0.33; Lin and Ji, 2000) and *P. elegans* (0.31; Du and Ji, 2001), the proportion of variation in clutch mass explained by female SVL was lower in *S. incognitus* (12%) than in *P. chinensis* (51%; Lin and Ji, 2000), *P. elegans* (46%; Du and Ji, 2001), *E. longicaudata* (42%; Sun *et al.*, 2012) and *S. modesta* (37%; Yang *et al.*, 2012), and the proportion

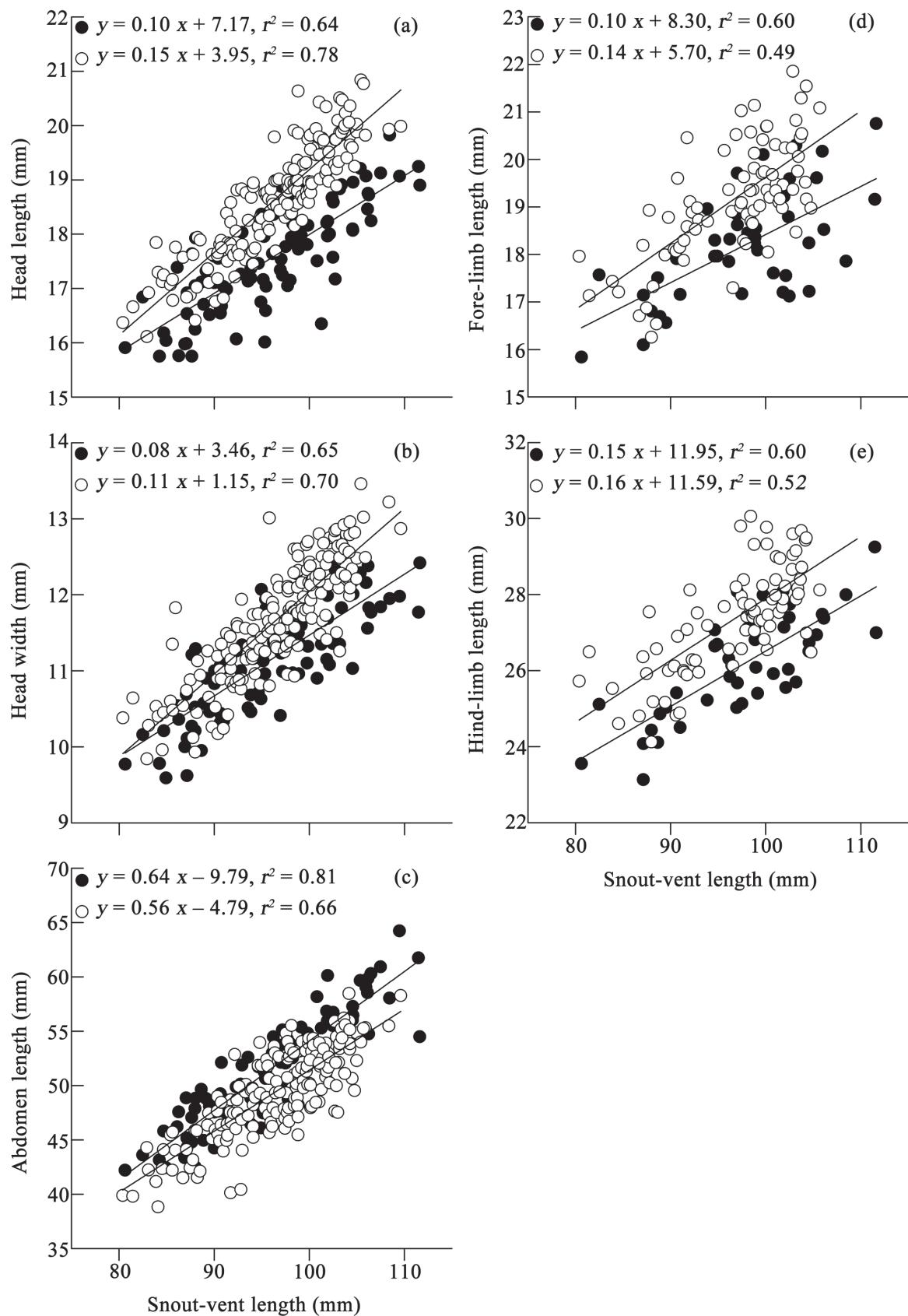


Figure 2 Linear regressions of head length (a), head width (b), abdomen length (c), fore-limb length (d) and hind-limb length (e) on SVL in adult *Sphenomorphus incognitus*. Filled circles: females; open circles: males.

of variation in clutch size explained by female SVL is lower in *S. incognitus* (18%) than in *P. chinensis* (52%; Lin and Ji, 2000), *S. modesta* (40%; Yang *et al.*, 2012), *P. elegans* (37%; Du and Ji, 2001) and *E. longicaudata* (35%; Sun *et al.*, 2012). These comparisons provide an inference that selection on increased maternal body size and thus increased body volume available to hold eggs is comparatively weak in *S. incognitus*.

3.3. Egg incubation and hatching phenotype

Embryonic stages at laying ranged from Dufaure and Hubert's (1961) stage 31 to 32, with a mean stage of 31.3. Embryonic stage at laying is a causal factor of inter- and intra-specific variation in incubation length in oviparous lizards (Wang *et al.*, 2013). However, incubation length at any given temperature may vary considerably among species that differ in phylogeny, egg size and/or distribution (Lin *et al.*, 2010; Li *et al.*, 2012, 2013; Sun *et al.*, 2013). Within sincid lizards, for example, the mean incubation length at 28 °C is much longer in *S. incognitus* (~40 d; Table 2) than in *S. modesta* (~20 d; Lu *et al.*,

2006) and *P. chinensis* (~24 d; Lu *et al.*, 2012, 2014; Shen *et al.*, 2017), although the mean DH stage at laying does not differ between *S. incognitus* and *S. modesta* (31.1; Lu *et al.*, 2006) and is about one stage earlier in *S. incognitus* than in *P. chinensis* (~32.5; Lu *et al.*, 2012, 2014; Shen *et al.*, 2017). In *Phrynocephalus* lizards the changeover from the DH stage 30 to 31 shortens the mean incubation length at 28 °C by 3 d (Wang *et al.*, 2013; Zeng *et al.*, 2013).

Eggs assigned to the five temperature treatments did not differ significantly in mean mass ($F_{4,42} = 2.44, P = 0.06$) or hatching success ($G = 2.62, df = 4, P > 0.50$). Hatching successes varied from 64% (16/25) in the 25 ± 5 °C treatment to 82% (9/11) in the 28 °C treatment, with a mean of 74% (Table 2). Within each treatment incubation length was independent of egg mass (linear regression analysis: all $P > 0.20$). Mean values for incubation length differed among the five treatments ($F_{4,42} = 45.62, P < 0.0001$). For eggs incubated at constant temperatures, the mean incubation length was shortened by 22.0 and 13.2 d for every 3 °C increase from 22–28 °C (Table 2).

Table 1 Reproductive traits of female *Sphenomorphus incognitus* ($N = 25$).

	Mean	Standard error	Range
Snout-vent length (mm)	96.4	1.2	81.4–107.6
Postpartum body mass (g)	17.9	0.7	11.0–28.3
Clutch size	5.5	0.3	3–10
Egg mass (g)	0.8	0.03	0.6–1.5
Egg length (mm)	14.8	0.2	11.9–17.2
Egg width (mm)	9.6	0.2	8.6–12.4
Clutch mass (g)	4.3	0.3	2.0–7.4
Relative clutch mass	0.25	0.02	0.1–0.5

Table 2 Hatching success and descriptive statistics (expressed as mean ± SE and range) for egg mass at laying (initial egg mass), incubation length and wet body mass and morphology of hatching *Sphenomorphus incognitus* from eggs incubated under five thermal regimes.

	Constant temperatures (°C)			Fluctuating temperatures (°C)	
	22	25	28	25 ± 3	25 ± 5
<i>N</i>	9	20	9	22	16
Initial egg mass (g)	0.72 ± 0.03 0.58-0.91	0.82 ± 0.02 0.68-0.97	0.74 ± 0.03 0.59-0.89	0.80 ± 0.03 0.67-1.04	0.82 ± 0.04 0.65-1.08
Hatching success (%)	69.2 (9/13) 75.5 ± 1.20	76.9 (20/26) 53.5 ± 2.05	81.8 (9/11) 40.3 ± 0.54	78.6 (22/28) 52.9 ± 2.25	64.0 (16/25) 43.7 ± 2.26
Incubation length (d)	68.0-79.5 30.6 ± 0.34	45.5-72.0 30.6 ± 0.30	38.0-43.0 30.3 ± 0.31	45.0-73.5 30.3 ± 0.55	35.0-53.5 30.0 ± 0.38
Snout-vent length (mm)	29.4-32.8 0.78 ± 0.03	29.4-32.3 0.78 ± 0.03	29.1-31.6 0.72 ± 0.03	27.7-32.7 0.72 ± 0.03	28.4-31.3 0.72 ± 0.03
Body mass (g)	0.66-0.96 14.1 ± 0.28	0.62-0.90 14.0 ± 0.30	0.58-0.84 14.0 ± 0.25	0.56-0.87 14.1 ± 0.33	0.58-0.87 13.2 ± 0.28
Abdomen length (mm)	13.1-16.6 8.1 ± 0.08	13.1-16.3 7.9 ± 0.12	12.7-15.1 7.9 ± 0.10	12.4-15.3 8.0 ± 0.089	12.4-14.2 7.9 ± 0.10
Head length (mm)	7.8-8.7 5.9 ± 0.06	7.0-8.4 5.7 ± 0.06	7.5-8.4 5.7 ± 0.13	7.7-8.4 5.5 ± 0.11	7.5-8.3 5.7 ± 0.11
Head width (mm)	5.5-6.2 5.3-6.1	5.0-6.2 5.0-6.2	5.1-6.1 5.1-6.1	5.2-6.1 5.2-6.1	

This pattern of thermal sensitivity of incubation length is consistent with earlier studies on turtles (Ji *et al.*, 2003, Du *et al.*, 2007, 2010), lizards (Ji and Braña, 1999; Lin *et al.*, 2007; Wang *et al.*, 2013; Shen *et al.*, 2017), snakes (Ji and Du, 2001; Lin *et al.*, 2005; Lin *et al.*, 2010) and crocodiles (Piña *et al.*, 2003; Charrua, 2012) where incubation length decreases at an ever decreasing rate as temperature increases across the range where successful embryonic development can take place, explaining why eggs take a longer time to hatch at fluctuating temperatures than at constant temperatures with the same mean in some species (Shine, 2004a; Hao *et al.*, 2006; Braña and Ji, 2007; Les *et al.*, 2007; Lu *et al.*, 2009; Li *et al.*, 2012). However, contrast to what was expected the fluctuating temperature treatments result in shorter incubation lengths relative to constant temperatures in *S. incognitus*. This suggests that, as in *Bassiana duperreyi* (Shine, 2004b), *Lycaena tityrus* (Fischer *et al.*, 2011), *Naja atra* (Lin *et al.*, 2008) and *Xenochrophis piscator* (Lu *et al.*, 2009), incubation at stable temperatures may lead to delayed hatching in *S. incognitus*.

Incubation temperatures higher than 28 °C substantially reduce hatching success and adversely affect hatchling phenotypes in forest skinks (Lu *et al.*, 2006; Li *et al.*, 2012). Here we found that hatchlings from eggs incubated at 25 ± 5 °C did not differ from those from eggs incubated under other four thermal regimes in any examined trait after accounting for egg mass at laying (ANCOVA; all $P > 0.19$; Table 2). This finding is overall consistent with that reported for a wide range of reptile taxa, including turtles (*Pelodiscus sinensis*: Du and Ji, 2003; Ji *et al.*, 2003), lizards (*E. argus*: Hao *et al.*, 2006; *Heteronotia binoei*: Andrewartha *et al.*, 2010; *Lacerta agilis*: Li *et al.*, 2013; *P. chinensis*: Chen *et al.*, 2003) and snakes (*Rhabdophis tigrinus lateralis*: Chen and Ji, 2002; *Ptyas mucosus*: Lin and Ji, 2004; *N. atra*: Lin *et al.*, 2008; *X. piscator*: (Lu *et al.*, 2009). In all these species, incubation temperature has no role in modifying hatchling traits as long as eggs are not exposed to extreme temperatures for prolonged periods of time.

4. Conclusions

Sphenomorphus incognitus is a morphologically, zoogeographically and taxonomically well known species, but its ecology and biology remain sparsely studied. Here we used adults collected from South China to study sexual dimorphism, female reproduction and egg incubation in this species. From this study we know the following. First, the skink is a sexually monomorphic species in

terms of adult SVL but shows sexual dimorphism in head size, abdomen length and limb length, with males being larger in head size (both length and width), longer in fore- and hind-limb lengths and shorter in abdomen length than females of the same SVL. Second, females larger than 80 mm SVL lay a single clutch of 3–10 eggs per breeding season from early May to mid-August, with larger females generally laying more (but not always larger) eggs per clutch than do smaller ones. Third, the positive relationship between clutch mass and female SVL is not significant, and the offspring size-number trade-off does not exist in *S. incognitus*. Fourth, embryonic stages at laying range from Dufaure and Hubert's (1961) stage 31 to 32, and the mean incubation length at a given temperature is much longer in *S. incognitus* compared to *S. modesta* with nearly the same embryonic stage at laying. Last, eggs of *S. incognitus* incubated at fluctuating temperatures take a shorter time to hatch than those incubated at stable temperatures with the same mean, and incubation temperature has no role in modifying hatchling morphology as long as eggs are not exposed to extreme temperatures for prolonged periods of time.

Acknowledgements Our experimental procedures complied with the current laws on animal welfare and research in China. We thank Yijing CHEN, Kun GUO, Min SHAO, Yanqing WU, Guangzheng ZHANG and Liang ZHANG for assistance during the research. For funding, we thank the National Natural Science Foundation of China (31470471), the Priority Academic Development Program of Jiangsu Higher Education Institutions and the Innovation of Graduate Student Training Project of Jiangsu Province (KYLX15_0737).

References

- Andrewartha S. J., Mitchell N. J., Frappell P. B.** 2010. Does incubation temperature fluctuation influence hatchling phenotypes in reptiles? A test using parthenogenetic geckos. *Physiol Biochem Zool*, 83: 597–607
- Auffenberg W., Auffenberg T.** 1989. Reproductive patterns in sympatric Philippine skinks (Sauria: Scincidae). *Bull FL State Mus Biol Sci Ser*, 34: 201–247
- Braña F.** 1996. Sexual dimorphism in lacertid lizards: male head increase vs female abdomen increase. *Oikos*, 75: 511–523
- Braña F., Ji X.** 2007. The selective basis for increased egg retention: Early incubation temperature determines hatchling phenotype in wall lizards. *Biol J Linn Soc*, 91: 441–447
- Bulté G., Irschick D. J., Blouin-Demerset G.** 2008. The reproductive role hypothesis explains trophic morphology dimorphism in the northern map turtle. *Funct Ecol*, 22: 824–830
- Charrua P.** 2012. Microclimate of American crocodile nests in Banco Chinchorro biosphere reserve, Mexico: Effect on

incubation length, embryos survival and hatchlings sex. *J Therm Biol*, 37: 6–14

Chen H. L., Ji X. 2002. The effects of thermal environments on duration of incubation, hatching success and hatchling traits in a colubrid snake *Rhabdophis tigrinus lateralis* (Boie). *Acta Ecol Sin*, 22: 1850–1858

Chen S. Y., Bi J. H., He Z. C., Li S. R., Liu R., Wang Y., Zhao X. 2015. Sexual dimorphism and reproductive output of *Eremias argus* from Ordos. *Chin J Zool*, 50: 214–220

Chen X. J., Lin Z. H., Ji X. 2003. Further studies on influence of temperature on egg incubation in the Chinese skink, *Eumeces chinensis*. *Zool Res*, 24: 21–25

Chen Z. Q., Wei H. H., Liu J. L., Wu Y. K., Le X. G., Cheng S. L., Guo H. X., Ding G. H. 2017. New record of *Sphenomorphus incognitus* from Zhejiang and Jiangxi provinces. *Sichuan J Zool*, 36: 479–480

Cooper W. E., Vitt L. J. 1989. Sexual dimorphism of head and body size in an iguanid lizard: paradoxical results. *Am Nat*, 133: 729–735

Cox R. M., Skelly S. L., John-Alder H. B. 2003. A comparative test of adaptive hypothesis for sexual size dimorphism in lizards. *Evolution*, 57: 1653–1669

Du W. G., Hu L. J., Lu J. L., Zhu L. J. 2007. Effects of incubation temperature on embryonic development rate, sex ratio and post-hatching growth in the Chinese three-keeled pond turtle, *Chinemys reevesii*. *Aquaculture*, 272: 747–753

Du W. G., Ji X. 2001. Growth, sexual dimorphism and female reproduction of blue-tailed skinks, *Eumeces elegans*. *Zool Res*, 22: 279–286

Du W. G., Ji X. 2003. The effects of incubation thermal environments on size, locomotor performance and early growth of hatchling soft-shelled turtles, *Pelodiscus sinensis*. *J Therm Biol*, 28: 279–286

Du W. G., Wang L., Shen J. W. 2010. Optimal temperatures for egg incubation in two geoemydid turtles: *Ocadia sinensis* and *Mauremys mutica*. *Aquaculture*, 305: 138–142

Dufaure J. P., Hubert J. 1961. Table de développement du lézard vivipare: *Lacerta (Zootoca) vivipara* Jacquin. *Arch Anat Microsc Morphol Exp*, 50: 309–328

Fischer K., Kölzow N., Höltje H., Karl I. 2011. Assay conditions in laboratory experiments: Is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia*, 166: 23–33

Hao Q. L., Liu H. X., Ji X. 2006. Phenotypic variation in hatchling Mongolian racerunners (*Eremias argus*) from eggs incubated at constant versus fluctuating temperatures. *Acta Zool Sin*, 52: 1049–1057

He N., Wu Z. J., Cai F. J., Wang Z. X., Yu H., Huang C. M. 2011. Sexual dimorphism of *Shinisaurus crocodilurus*. *Chin J Ecol*, 30: 7–11

Hernández-Salinas U., Ramírez-Bautista A., Pavón N. P., Pacheco L. F. R. 2014. Morphometric variation in island and mainland populations of two lizard species from the Pacific Coast of Mexico. *Rev Chil Hist Nat*, 87: 21

Hews, D. K. 1990. Examining hypotheses generated by field measures of sexual selection on male lizards, *Uta palmeri*. *Evolution*, 44: 1956–1966

Huang Q. Y. 1999. Scincidae. In Zhao E. M., Zhao K. T., Zhou K. Y. (Eds.), *Fauna Sinica, Reptilia*, Vol. 2. Beijing: Science Press, 271–360

Huang W. S. 1996. Reproductive cycles and sexual dimorphism in the viviparous skink, *Sphenomorphus indicus* (Sauria: Scincidae), from Wushe, Central Taiwan. *Zool Stud*, 35: 55–61

Huang W. S. 1997. Reproductive cycle of the skink, *Sphenomorphus taiwanensis*, in central Taiwan. *J Herpetol*, 31: 287–290

Huang W. S. 1998. Sexual size dimorphism and microhabitat use of two sympatric lizards, *Sphenomorphus taiwanensis* and *Takydromus hsuehshanensis*, from the central highlands of Taiwan. *Zool Stud*, 37: 303–308

Huang W. S. 2010. Ecology and reproductive characteristics of the skink *Sphenomorphus incognitus* on an East Asian Island, with comments on variations in clutch size with reproductive modes in *Sphenomorphus*. *Zool Stud*, 49: 779–788

Ji X., Braña F. 1999. Influence of thermal and hydric environments on embryonic use of energy and nutrients, and hatchling traits, in the wall lizards (*Podarcis muralis*). *Comp Biochem Physiol*, 124A: 205–213

Ji X., Chen F., Du W. G., Chen H. L. 2003. Incubation temperature affects hatchling growth but not sexual phenotype in the Chinese soft-shelled turtle *Pelodiscus sinensis*. *J Zool*, 261: 409–416

Ji X., Du W. G. 2000. Sexual dimorphism in body size and head size and female reproduction in a viviparous skink, *Sphenomorphus indicus*. *Zool Res*, 21: 349–354

Ji X., Huang H. Y., Hu X. Z., Du W. G. 2002. Geographic variation in female reproductive characteristics and egg incubation in the Chinese skink, *Eumeces chinensis*. *Chin J Appl Ecol*, 13: 680–684

Ji X., Lin C. X., Lin L. H., Qiu Q. B., Du Y. 2007. Evolution of viviparity in warm-climate lizards: An experimental test of the maternal manipulation hypothesis. *J Evol Biol*, 20: 1037–1045

Ji X., Lin L. H., Lin C. X., Qiu Q. B., Du Y. 2006. Sexual dimorphism and female reproduction in the many-lined sun skink (*Mabuya multifasciata*) from China. *J Herpetol*, 40: 353–359

Ji X., Lin L. H., Luo L. G., Lu H. L., Gao J. F., Han J. 2006. Gestation temperature affects sexual phenotype, morphology, locomotor performance and growth of neonatal brown forest skink, *Sphenomorphus indicus*. *Biol J Linn Soc*, 88: 453–463

Ji X., Qiu Q. B., Diong C. H. 2002. Sexual dimorphism and female reproductive characteristics in the oriental garden lizard, *Calotes versicolor* from a population in Hainan, southern China. *J Herpetol*, 36: 1–8

Ji X., Wang Y. Z., Wang Z. 2009. New species of *Phrynocephalus* (Squamata, Agamidae) from Qinghai, Northwest China. *Zootaxa*, 1988: 61–68

Ji X., Zhang C. H. 2001. Effects of thermal and hydric environments on incubating eggs, hatching success, and hatchling traits in the Chinese skink (*Eumeces chinensis*). *Acta Zool Sin*, 47: 250–259

Ji X., Zhou W. H., He G. B., Zhang X. D. 1998. Sexual dimorphism and reproduction in the grass lizard, *Takydromus septentrionalis*. *Russ J Herpetol*, 5: 44–48

Kratochvíl L., Fokt M., Rehák I., Frynta D. 2003. Misinterpretation of character scaling: A tale of sexual dimorphism in body shape of common lizards. *Can J Zool*, 81:

1112–1117

Lau M. 2005. The occurrence of *Sphenomorphus incognitus* in Hong Kong with notes on its diagnostic features and distribution. *Porcupine*, 32: 9–10

Les H. L., Paitz R. T., Bowden R. M. 2007. Experimental test of the effects of fluctuating incubation temperatures on hatching phenotype. *J Exp Zool A*, 307: 274–280

Li H., Ding G. H., Zhou Z. S., Ji X. 2013. Fluctuations in incubation temperature affect incubation duration but not morphology, locomotion and growth of hatchlings in the sand lizard *Lacerta agilis* (Lacertidae). *Acta Zool (Stockholm)*, 94: 11–18

Li H., Ji X., Qu Y. F., Gao J. F., Zhang L. 2006. Sexual dimorphism and female reproduction in the multi-ocellated racerunner, *Eremias multiocellata* (Lacertidae). *Acta Zool Sin*, 52: 250–255

Li H., Wang Z., Chen C., Ji X. 2012. Does the variance of incubation temperatures always constitute a selective force for the origin of reptilian viviparity? *Curr Zool*, 58: 812–819

Lin C. X., Du Y., Qiu Q. B., Ji X. 2007. Relatively high but narrow incubation temperatures in lizards depositing eggs in warm and thermally stable nests. *Acta Zool Sin*, 53: 437–445

Lin L. H., Li H., An H., Ji X. 2008. Do temperature fluctuations during incubation always play an important role in shaping the phenotype of hatching reptiles? *J Therm Biol*, 33: 193–199

Lin L. H., Ma X. M., Li H., Ji X. 2010. Phenotypic variation in hatching Chinese ratsnakes (*Zaocys dhumnades*) from eggs incubated at constant temperatures. *J Therm Biol*, 35: 28–33

Lin Z. H. 2004. Sexual dimorphism in head and body size and the growth during reproductive period in the lizard, *Japalura splendida*. *Sichuan J Zool*, 23: 277–280

Lin Z. H., Ji X. 2000. Food habits, sexual dimorphism and female reproduction of the skink (*Eumeces chinensis*) from a Lishui population in Zhejiang. *Acta Ecol Sin*, 20: 304–310

Lin Z. H., Ji X. 2004. Reproductive output and effects of incubation thermal environments on hatching phenotypes of mucous rat snakes *Ptyas mucosus*. *Acta Zool Sin*, 50: 541–550

Lin Z. H., Ji X., Luo L. G., Ma X. M. 2005. Incubation temperature affects hatching success, embryonic expenditure of energy and hatching phenotypes of a prolonged egg-retaining snake, *Deinagkistrodon acutus* (Viperidae). *J Therm Biol*, 30: 289–297

Linkem C. W., Diesmos A. C., Brown R. M. 2011. Molecular systematics of the Philippine forest skinks (Squamata: Scincidae: *Sphenomorphus*): Testing morphological hypotheses of interspecific relationships. *Zool J Linn Soc*, 163: 1217–1243

Liu Y., Shi L. 2009. Ontogenetic shifts of sexual dimorphism in *Phrynocephalus grumgrzimailoi* (Agamidae). *Sichuan J Zool*, 28: 710–713

Lu H., Gao J. F., Ma X. H., Lin Z. H., Ji X. 2012. Tail loss affects fecundity but not offspring traits in the Chinese skink, *Eumeces chinensis*. *Curr Zool*, 58: 228–235

Lu H. L., Hu R. B., Ji X. 2009. The variance of incubation temperatures does not affect the phenotype of hatchlings in a colubrid snake, *Xenochrophis piscator* (Colubridae). *J Therm Biol*, 34: 138–143

Lu H. L., Ji X., Lin L. H., Zhang L. 2006. Relatively low upper threshold temperature in lizards using cool habitats. *J Therm Biol*, 31: 256–261

Lu H. L., Lin Z. H., Li H., Ji X. 2014. Geographic variation in hatching size in an oviparous skink: Effects of maternal investment and incubation thermal environment. *Biol J Linn Soc*, 113: 283–296

Mouton P. F. N., Van Wyk J. H. 1993. Sexual dimorphism in cordylid lizards: A case study of the Drakensberg crag lizard, *Pseudocordylus melanotus*. *Can J Zool*, 71: 1715–1723

Nguyen T. Q., Schmitz A., Nguyen T. T., Orlov N. L., Böhme W., Ziegler T. 2011. Review of the genus *Sphenomorphus* Fitzinger, 1843 (Squamata: Sauria: Scincidae) in Vietnam, with description of a new species from northern Vietnam and southern China and the first record of *Sphenomorphus mimicus* Taylor, 1962 from Vietnam. *J Herpetol*, 45: 145–154

Nguyen T. Q., Tran T. T., Nguyen T. T., Böhme W., Ziegler T. 2012. First record of *Sphenomorphus incognitus* (Thompson, 1912) (Squamata: Scincidae) from Vietnam with some notes on natural history. *Asian Herpetol Res*, 3: 147–150

Olsson M., Shine R. 1997. The limits to reproductive output: offspring size versus number in the sand lizard (*Lacerta agilis*). *Am Nat*, 149: 179–188

Olsson M., Shine R., Wapstra E., Ujvari B., Madsen T. 2002. Sexual dimorphism in lizard body shape: The roles of sexual selection and fecundity selection. *Evolution*, 56: 1538–1542

Piña C. I., Larriera A., Cabrera M. R. 2003. Effect of incubation temperature on incubation period, sex ratio, hatching success, and survivorship in *Caiman latirostris* (Crocodylia, Alligatoridae). *J Herpetol*, 37: 199–202

Pincheira-Donoso D., Tregenza T. 2011. Fecundity selection and the evolution of reproductive output and sex-specific body size in the *Liolaemus* lizard adaptive radiation. *Evol Biol*, 38: 197–207

Qu Y. F., Gao J. F., Mao L. X., Ji X. 2011. Sexual dimorphism and female reproduction in two sympatric toad-headed lizards, *Phrynocephalus frontalis* and *P. versicolor* (Agamidae). *Anim Biol*, 61: 139–151

Ramírez-Bautista A., Cruz-Elizalde R., Hernández-Salinas U., Lozano A., Grummer J. A. 2017. Reproductive trait variation in the *Sceloporus scalaris* species group (Squamata: Phrynosomatidae) from the Transvolcanic Belt, Mexico. *Biol J Linn Soc*, 122: 838–849

Reeve J. P., Fairbairn D. J. 2001. Predicting the evolution of sexual size dimorphism. *J Evol Biol*, 14: 244–254

Shen W., Pei J. C., Lin L. H., Ji X. 2017. Effects of constant versus fluctuating incubation temperatures on hatching success, incubation length and hatching morphology in the Chinese skink (*Plestiodon chinensis*). *Asian Herpetol Res*, 8: 262–268

Shine R. 1992. Relative clutch mass and body shape in lizards and snakes: its reproductive investment constrained or optimized? *Evolution*, 46: 828–833

Shine R. 2004a. Incubation regimes of cold-climate reptiles: The thermal consequences of nest-site choice, viviparity and maternal basking. *Biol J Linn Soc*, 83: 145–155

Shine R. 2004b. Seasonal shifts in nest temperature can modify the phenotypes of hatching lizards, regardless of overall mean incubation temperature. *Funct Ecol*, 18: 43–49

Sun B. J., Li S. L., Xu X. F., Zhao W. G., Luo L. G., Ji X., Du W. G. 2013. Different mechanisms lead to convergence of

reproductive strategies in two lacertid lizards (*Takydromus wolteri* and *Eremias argus*). *Oecologia*, 172: 645–652

Sun Y. Y., Du Y., Yang J., Fu T. B., Lin C. X., Ji X. 2012. Is the evolution of viviparity accompanied by a relative increase in maternal abdomen size in lizards? *Evol Biol*, 39: 388–399

Tang X. S., Huang S. 2014. *Sphenomorphus incognitus* firstly found in Anhui province, China. *Chin J Zool*, 49: 609–612

Wang Z., Lu H. L., Ma L., Ji X. 2014. Viviparity in high altitude *Phrynocephalus* lizards is adaptive because embryos cannot fully develop without maternal thermoregulation. *Oecologia*, 174: 639–649

Wang Z., Ma L., Shao M., Ji X. 2013. Differences in incubation length and hatching morphology among five oviparous *Phrynocephalus* lizards (Agamidae) from China. *Asian Herpetol Res*, 4: 225–232

Xu D. D., Luo S. T., Liu W. H., Yao X. M., Wu H. X. 2014. The intersexual differences of sexual dimorphism, feeding habits and locomotor performance at different temperatures of southern grass lizard (*Takydromus sexlineatus*) in Zhaoqing, China. *Sichuan J Zool*, 33: 808–814

Xu X. F., Ji X. 2003. Ontogenetic shifts in sexual dimorphism in head size and food habits in the lacertid lizard, *Eremias brenchleyi*. *Chin J Appl Ecol*, 14: 557–561

Yang J., Sun Y. Y., Fu T. B., Xu D. D., Ji X. 2012. Selection for increased maternal body-volume does not differ between two *Scincella* lizards with different reproductive modes. *Zoology*, 115: 199–206

Zeng Z. G., Zhao J. M., Sun B. J. 2013. Life history variation among geographically close populations of the toad-headed lizard (*Phrynocephalus przewalskii*): Exploring environmental and physiological associations. *Acta Oecol*, 51: 28–33

Zhang Y. P., Ji X. 2000. Ontogenetic changes of sexual dimorphism in head size and food habit in grass lizard, *Takydromus septentrionalis*. *Zool Res*, 21: 181–186

Zhang Y. P., Ji X. 2004. Sexual dimorphism in head size and food habits in the blue-tailed skink, *Eumeces elegans*. *Acta Zool Sin*, 50: 745–752

Zhao W., Liu N. F. 2014. The proximate causes of sexual size dimorphism in *Phrynocephalus przewalskii*. *PLoS One*, 9: e85963